

Islet of Langerhans in Congenital Hyperinsulinism in Infancy are Disrupted and Show Decreased Expression of Collagen (IV) α 1 Chain in Basement Membranes

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Introduction and Objectives

Congenital hyperinsulinism of infancy (CHI) is the most common cause of severe hypoglycaemia in children. It mainly arises from mutations in ion channel genes which lead to inappropriate insulin secretion. Normal adult islets are encapsulated within a basement membrane (BM) which can fold into the islet forming a second layer around blood vessel BMs¹. However, it is also associated with marked changes in islet organization². Our aims were to determine the structure of the islet capsule and to examine the extracellular matrix composition of the islet capsule and blood vessels in tissue from patients with CHI and age-matched controls.

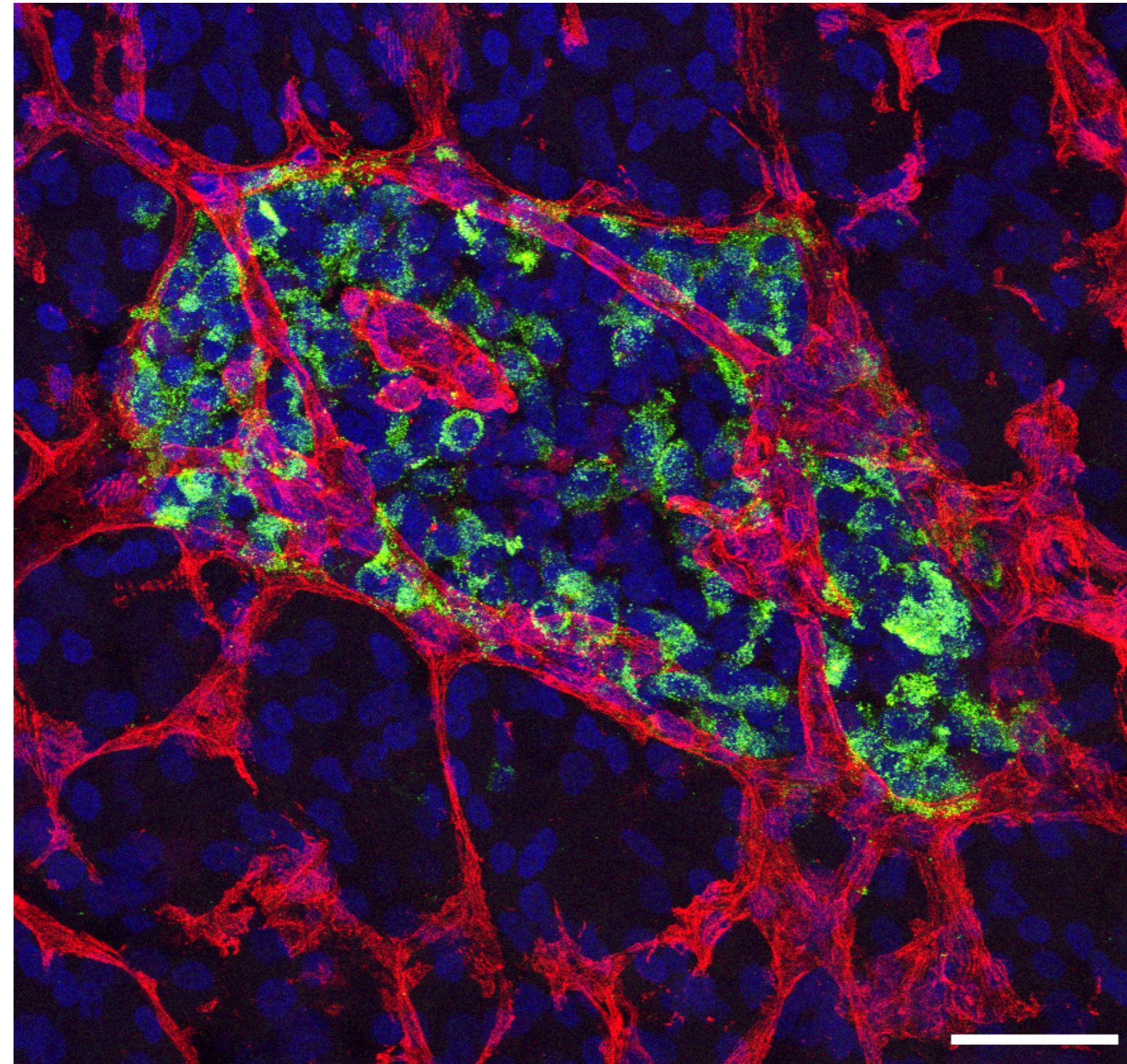


Fig. 1. Confocal micrograph of normal human islet. Collagen IV in islet BM (red) encapsulate all endocrine cells including β -cells (green). Nuclei (blue). Islet depth is 23.6 μ m. Scale bar represents 20 μ m.

Methods

Pancreas tissue was obtained from CHI patients following surgery and from autopsy specimens of age-matched control infants. Islet capsule and intra-islet blood vessels structures were demonstrated after staining diffuse CHI (CHI-D) (n=7, 2-13 months) tissue and control tissue with PicroSirius Red (PSR). Collagen distribution was quantified using ImageJ analysis after imaging the PSR-stained slide on a polarising microscope. Then, immunostaining was performed on CHI-D (n=4, 2-5 months), lesion and non-lesion focal CHI (CHI-F) (n=3, <3-4 months), atypical CHI (n=1, 3 years) and controls to examine the expression pattern of collagen (IV) α 1 chain (COL4A1) in islets and intra islet basement membranes. COL4A1 optical density (OD) from 10 randomly selected images per case were quantified using ImageJ software.

Results 1: The islet capsule is disturbed in CHI-D

In control tissue, islets were encapsulation within a clearly defined basement membrane (Panel A). However, in CHI-D (n=7, 2-13 months) more than 75% of islets were found to be poorly encapsulated (Panels B). This was significantly higher than found in in control islets (n=4, age 7 weeks-10 months).

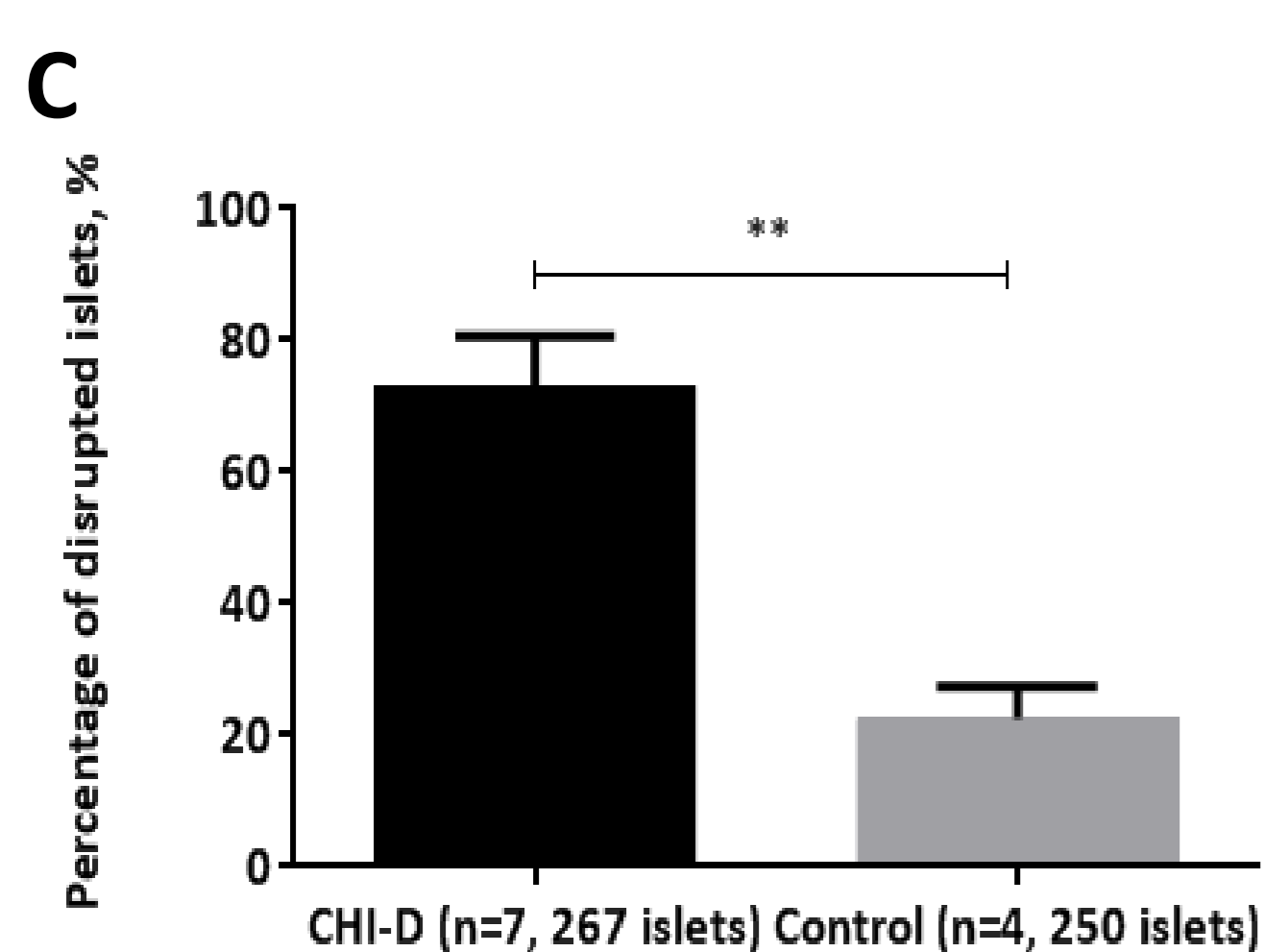
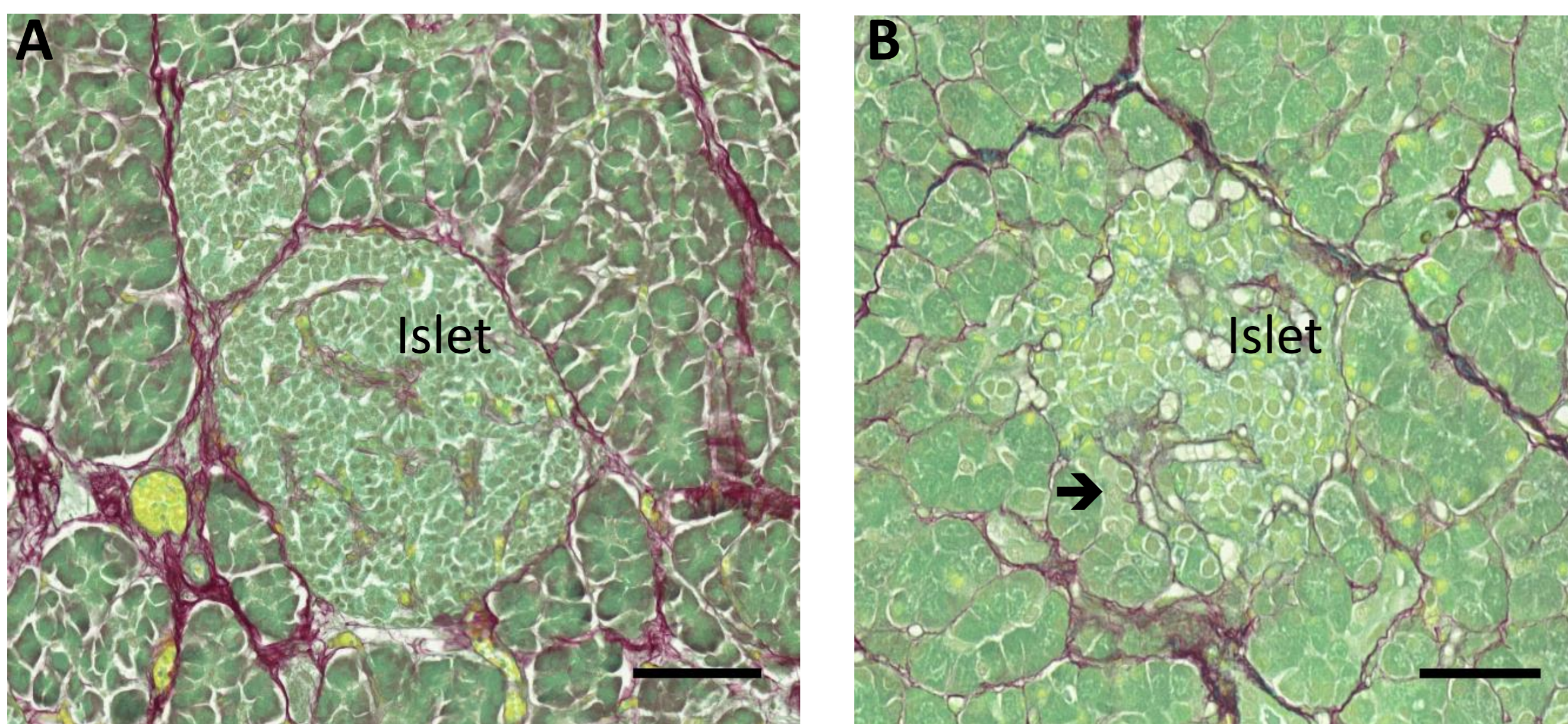


Fig. 2. Islet capsule organisation is disrupted in the CHI pancreas. Both tissues were stained with PicroSirius Red. Complete encapsulation in control tissue (A). Disturbed encapsulation in CHI-D (B) with exocrine-endocrine association (arrow). Percentages of the disrupted islets in CHI-D and controls (C). Scale bars represent 100 μ m. Unpaired t-test was used. Error bars show SEM, ** p \leq 0.01.

Results 2: COL4A1 expression is reduced in CHI

There was marked decrease in the expression of pancreatic COL4A1 in all CHI-D tissues (n=4, 2-5 months) compared to focal CHI (n=3, 3-12 months), atypical CHI (n=1, <3-4 months) and age matched controls. Optical density (OD) measurements of DAB staining showed that most CHI tissues have significantly lower collagen OD compared to the control.

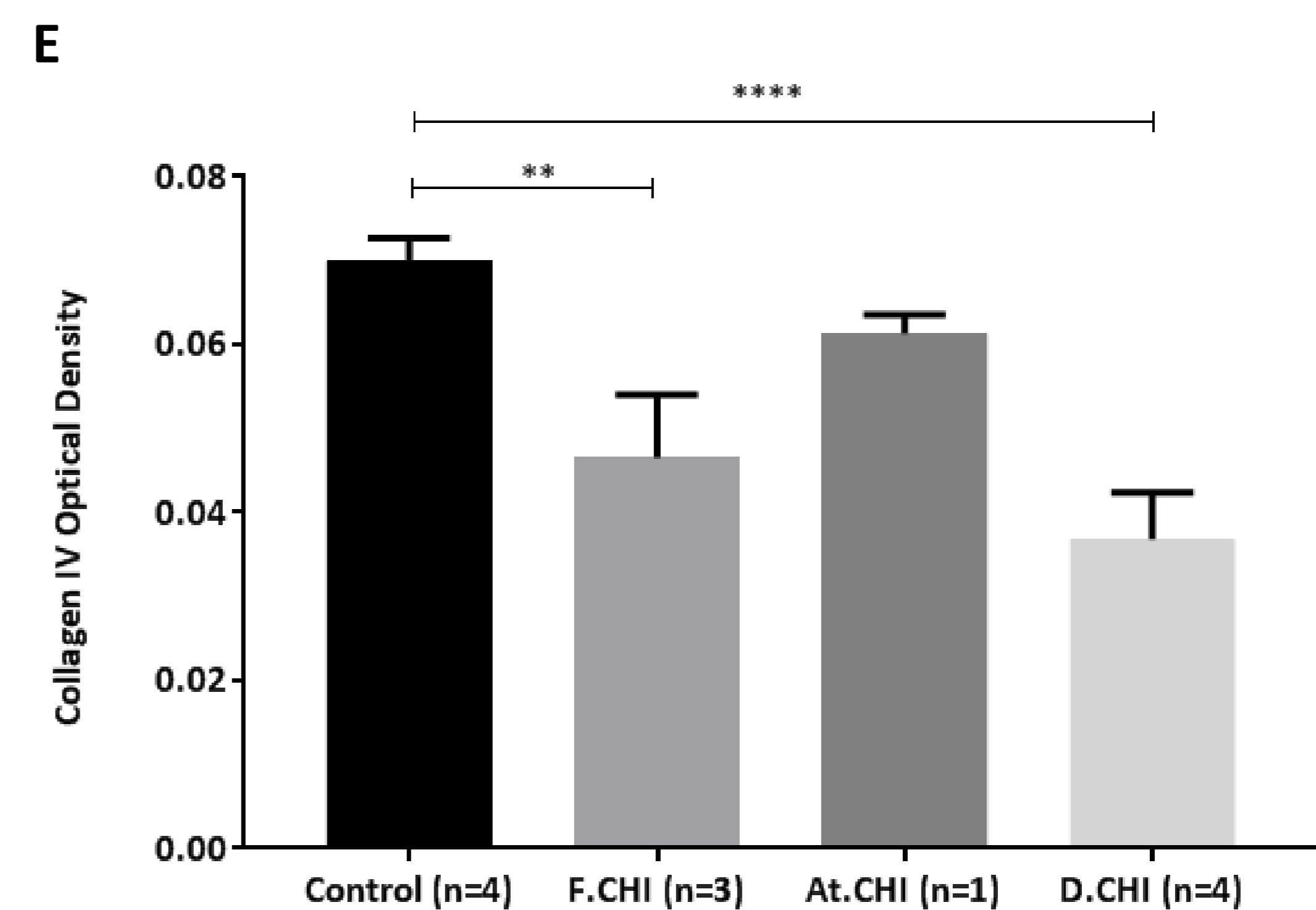
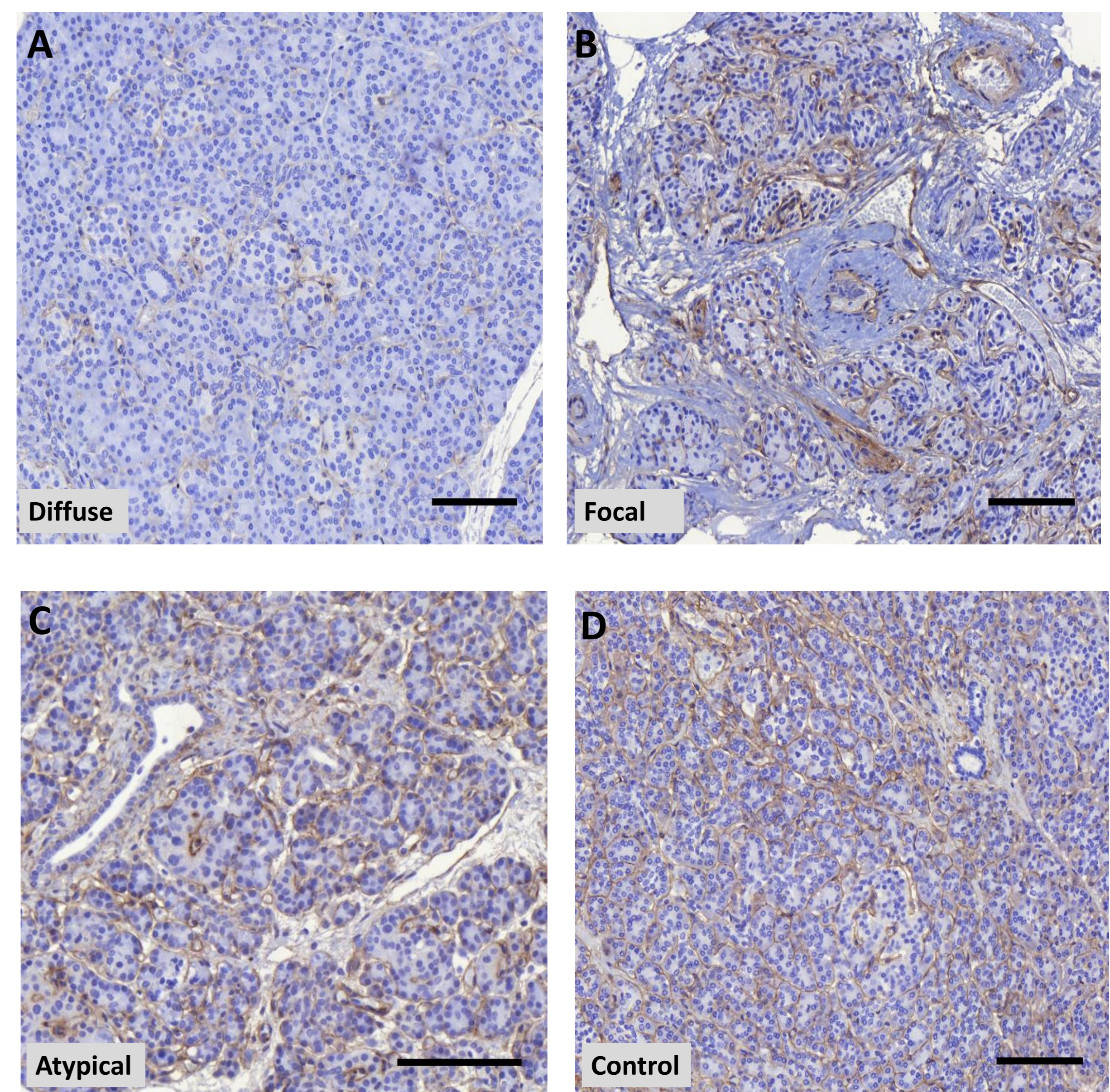


Fig. 3. COL4A1 protein expression is decreases in the CHI pancreas. Immunohistochemistry staining of COL4A1 in CHI-D (A), CHI-F (B), atypical CHI (C) and normoglycaemic control (D). Scale bars represent 100 μ m. In Panel E the estimated expression levels of COL4A1 have been determined from optical density measurements of DAB in control, focal CHI (F.CHI), atypical (At.CHI) and diffuse tissue (D.CHI). One-Way ANOVA with Dunnett's multiple comparison test was used. Error bars show SEM; ** p \leq 0.01, **** p \leq 0.0001.

Summary

Diffuse CHI is associated with disturbances in islet architecture and lower collagen content compared to age-matched control tissue. The low level of COL4A1 expression supports the possible involvement of the islet matrix in the pathogenesis of CHI.

References

- Virtanen et al., *Diabetologia* (2008) 51: 1181-1191.
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